Supporting Information

Baskin *et al.* 10.1073/pnas.0813234106

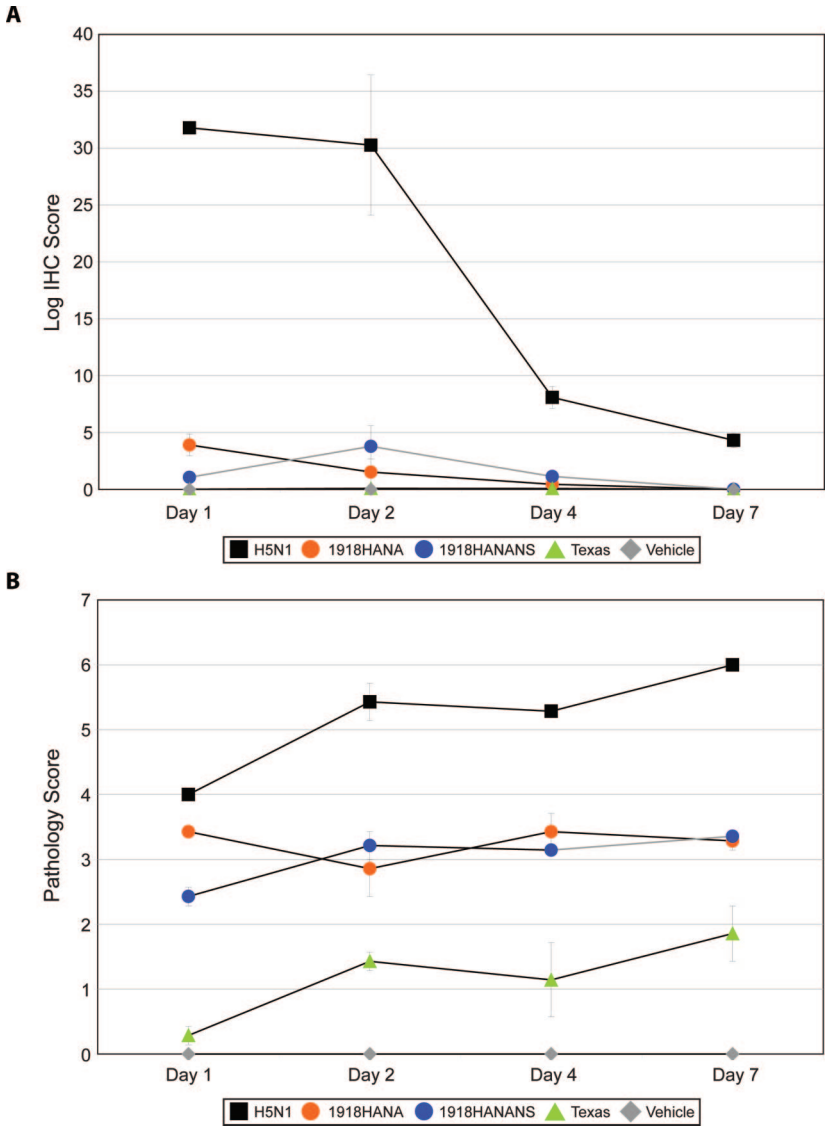

Fig. S1. (*A*) Lung viral antigen scoring. All scores are numbers of positive cells per ''N'' 40× objective fields; a minimum of 10 fields were counted for each specimen; when a lung appeared negative for influenza antigen, the entire specimen was scanned using a 20× objective to verify the finding. A 2-way ANOVA confirmed that the H5N1 group was statistically different from all of the others ($P < 0.001$). Pairwise comparisons (Student Newman–Keuls Method, $P \leq 0.05$) revealed that the H5N1 group was different from all of the others at every endpoint, with the exception of day 7. (*B*) The pathological changes in the lungs were scored on a scale of 0 to 6, taking into account the following features of the inflammation: degree and type of leukocyte infiltration (granulocytes, monocyte-macrophages, and lymphocytes), vascular reactions (leukocyte margination and endothelial cell hypertrophy) and/or leakage (fibrin transudation, hyaline membrane formation, and erythrocyte extravasation or frank hemorrhage), cellular necrosis/apoptosis, and exfoliation. Finally, repair was judged by the degree and extent of pneumocyte hypertrophy and hyperplasia, and fibrin-organization. The scores were: 0 = no apparent changes; 1 = minimal changes (including background ''noise''); 2 = mild inflammation and/or pneumocyte hypertrophy; 3 = moderate inflammation and/or pneumocyte hypertrophy; 4 = marked inflammation and/or pneumocyte hypertrophy; 5 = severe inflammation and/or pneumocyte hypertrophy affecting less than 50% of lung tissue examined; 6 = severe inflammation and/or pneumocyte hypertrophy affecting more than 50% of lung tissue examined. Multiple serial sections from each lung lobe were examined (5–25/lobe; 7 lobes). A 2-way ANOVA confirmed that the groups were statistically different from one another ($P < 0.001$), with the exception of the two 1918 reassortants. Pairwise comparisons (Student Newman–Keuls method, $P \leq 0.05$) revealed that the H5N1 group was different from all the others at every endpoint, with the exception of day 1 with the 1918HANA group. On other days, this latter could not be differentiated from 1918 HANANS. The Texas group was different from all of the others but the control on every endpoint. Note: Day 6 death in H5N1 group is illustrated as day 7 for simplicity.

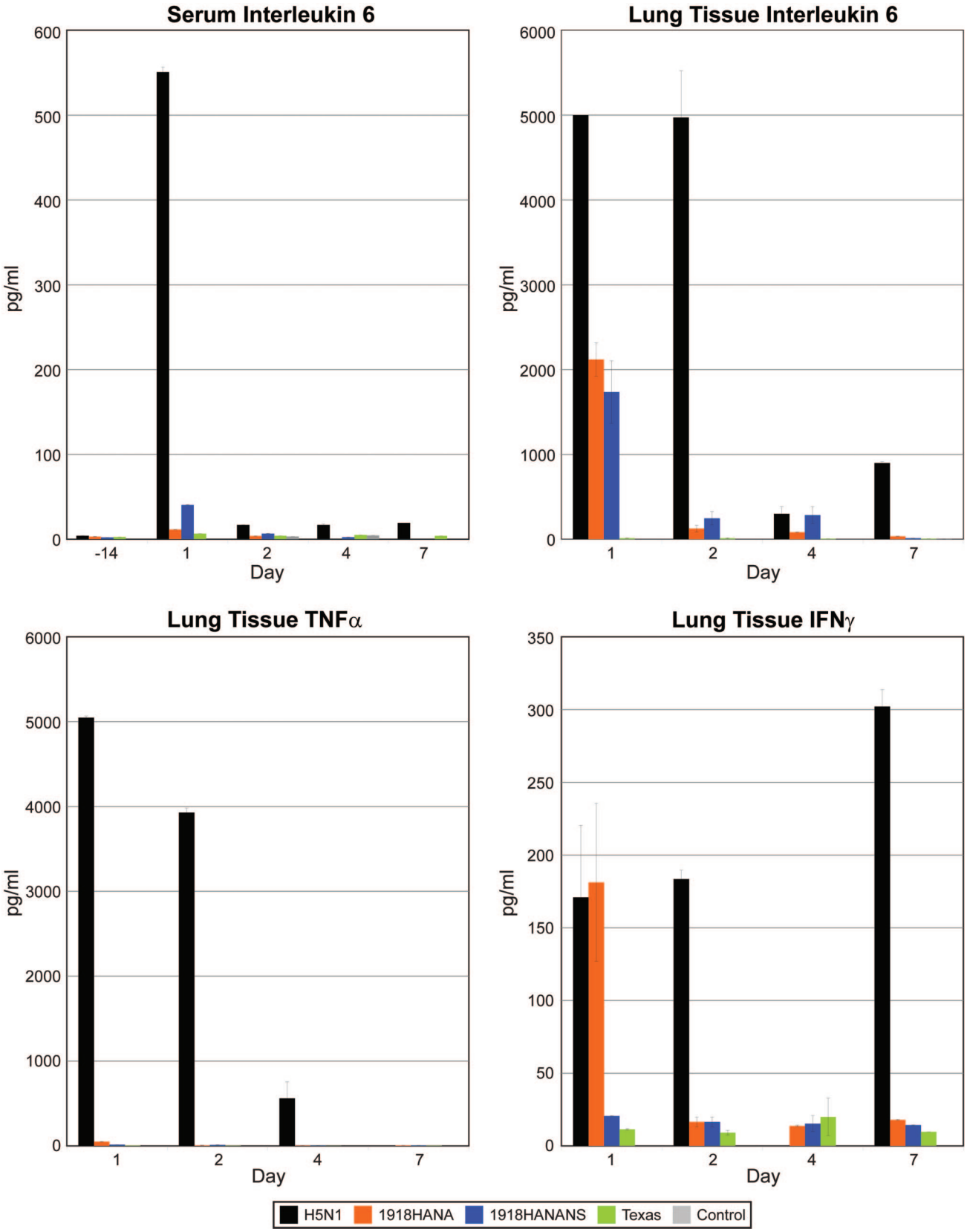

Fig. S2. Cytokine bead arrays demonstrate the dramatic elevation of serum and lung tissue IL-6, TNF-α, and IFN-γ in the H5N1 group as compared with 1918 reassortants and Texas groups. Note: No peripheral blood sample could be harvested at day 7 from the H5N1 animal that died on day 6 PI.

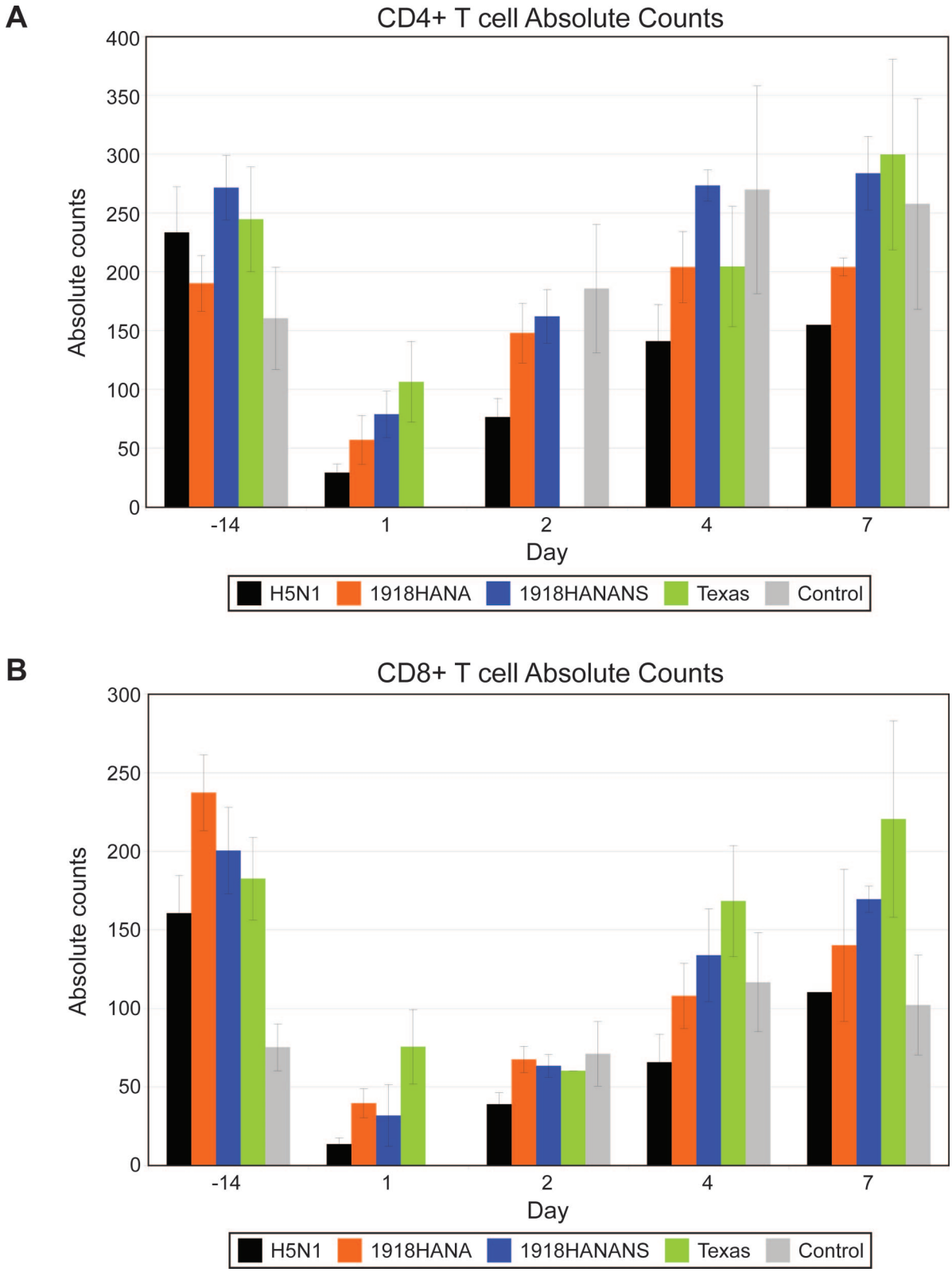

Fig. S3. Absolute average $CD4^+$ and $CD8^+$ cell counts, measured by FACS analysis, reveal dramatic and prolonged decrease of these cells in circulation after infection in all groups, but particularly in the H5N1 animals. Note: No peripheral blood sample could be harvested at day 7 from the H5N1 animal that died on day 6 PI.

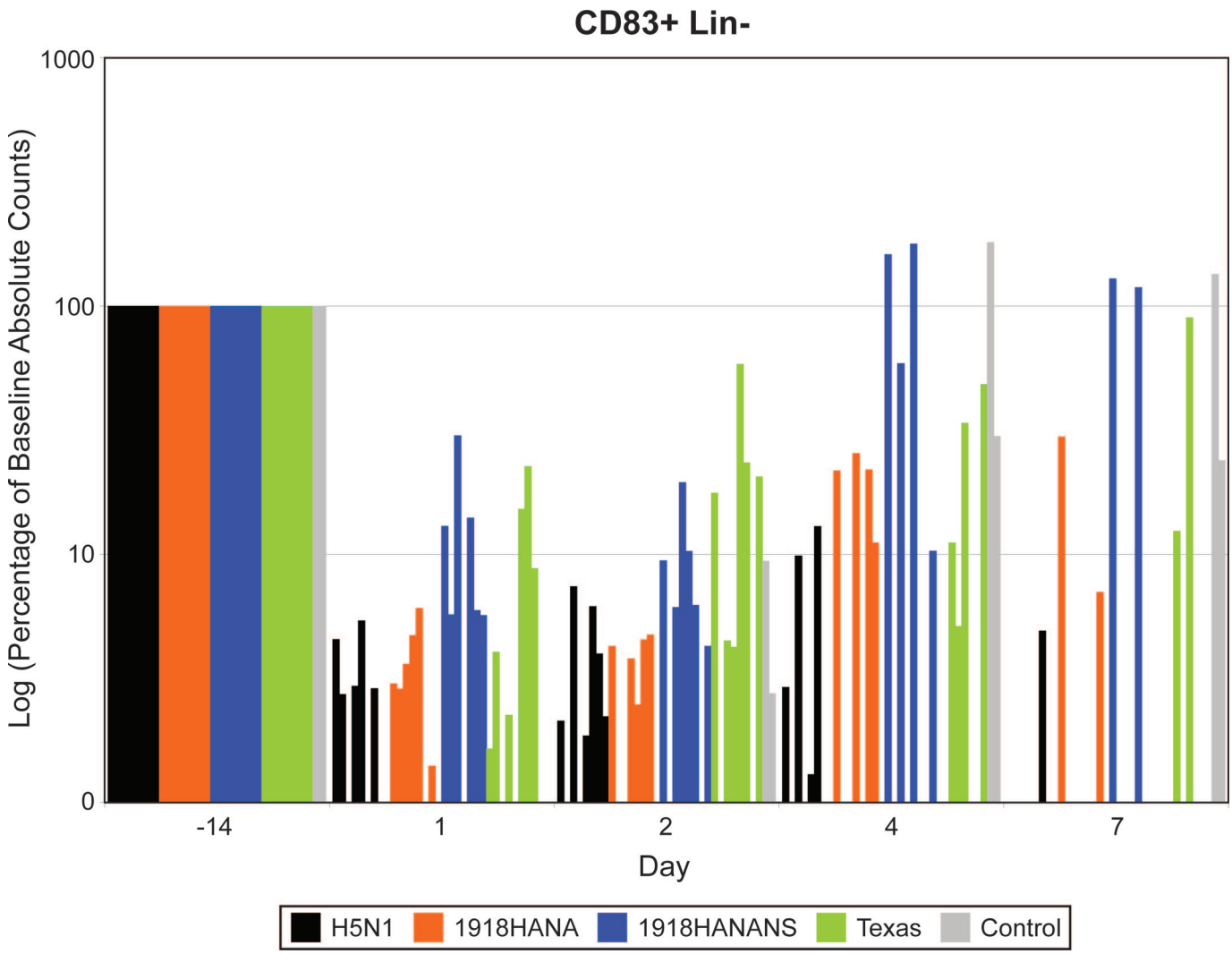

Fig. S4. Absolute individual CD83^{+} (Lin^{-}) cell counts, measured by FACS analysis, showed a moderate decrease in circulation in all groups. The data are shown as a percent of the baseline to allow the comparison of the progression of these counts between animals, which differed on day −14. Note: No peripheral blood sample could be harvested at day 7 from the H5N1 animal that died on day 6 PI.

Table S1. Clinical scoring system used in this experiment

Parameter	Degree of parameter	Possible score
Fever	Normal (<102.2 °F)	0
	Elevated temperature (102.2–104 °F)	3
	High temperature (>104 °F)	5
Posture/attitude	Normal	0
	Piloerection of body hair	1
	Decreased activity, decreasing normal behavior, piloerection	2
	Found dead	15
Respiration	Normal	0
	Increased or decreased; mild cough and clear nasal discharge	3
Appetite	Normal	0
	Decreased	2
	No stools	3
Weight loss	None or <5%	0
	>5%, <10%	2
	>10%	4
GI distress	Soft stools	2
	Vomiting	2
	Diarrhea	3

Animals were monitored and scored twice daily.